Table I

Cyclohexylamine (NH_2 equatorial)	Composition of product		Cyclohexanol produced (OH equatorial)	Ref.
	Cyclohexene	Cyclohexanol		
"trans"-3-Methylcyclohexylamine[1,2]			cis-3-Methylcyclohexanol[6]	2
trans-4-Methylcyclohexylamine[2]			trans-4-Methylcyclohexanol[2] 60% yield	3
trans-4-Phenylcyclohexylamine[3]			trans-4-Phenylcyclohexanol[3] 53% yield	3
trans-4-Cyclohexylcyclohexylamine[3]			trans-4-Cyclohexylcyclohexanol 49% yield	3
trans-4-t-Butylcyclohexylamine[3]			trans-4-t-Butylcyclohexanol[3] 55% yield	3
Menthylamine	Little	Mostly	Menthol[7]	8
iso-Menthylamine	Little	Mostly	iso-Menthol[7]	8
Carvomenthylamine[4]	15%	85%	Carvomenthol[4]	9
trans-α-Decalylamine[5], m.p. – 1°	nil	100%	trans-α-Decalol[5], m.p. 63°	5
trans-β-Decalylamine[5], m.p. 15°	nil	100%	trans-β-Decalol[5], m.p. 75°	5

[1] Conformational analysis indicates that "trans"-1,3-methylcylohexylamine should actually be termed cis.
[2] M. M. CLAUDON, Bull. Soc. chim. France, *1950*, 627.
[3] D. V. NIGHTINGALE, J. D. KERR, J. A. GALLAGHER, and M. MAIENTHAL, J. Org. Chem. *17*, 1017 (1952).
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[7] D. H. R. BARTON, Exper. *6*, 316 (1950).
[8] J. READ, A. M. COOK, and M. I. SHANNON, J. Chem. Soc. *1926*, 2223.
[9] R. G. JOHNSTON and J. READ, J. Chem. Soc. *1935*, 1138.

Table II

Cyclohexylamine (NH_2 polar)	Composition of product		Cyclohexanol produced	Ref.
	Cyclohexene	Cyclohexanol		
"cis"-3-Methylcyclohexylamine[1]			45% WALDEN inversion	2
cis-4-Methylcyclohexylamine[5]			25% WALDEN inversion	2
neo-Menthylamine	81%	19%	Mixture of epimers	6
neo-iso-Menthylamine	80%	20%	Mixture of epimers	6
neo-Carvomethylamine[3]	80%	20%	60% WALDEN inversion	3
trans-α-Decalylamine[4], m.p. – 18°	70%	30%	90% WALDEN inversion	4
trans-β-Decalylamine[4], m.p. – 47°	70%	30%	90% WALDEN inversion	4

[1] Conformational analysis indicates that "Cis"-1,3-methylcylohexylamine should actually be termed trans.
[2] M. M. CLAUDON, Bull. Soc. chim. France, *1950*, 627.
[3] A. K. BOSE, Exper. *8*, 458 (1952).
[4] W. HÜCKEL, Ann. Chem. *533*, 1 (1938).
[5] D. V. NIGHTINGALE, J. D. KERR, J. A. GALLAGHER, and M. MAIENTHAL, J. Org. Chem. *17*, 1017 (1952).
[6] J. READ and G. J. ROBERTSON, J. Chem. Soc. *1927*, 2168.
[7] R. G. JOHNSTON and J. READ, J. Chem. Soc. *1935*, 1138.

The reaction of nitrous acid with cyclohexylamines possesses diagnostic value and promises to be useful for conformation determination[1].

A. K. BOSE

Applied Chemistry Department, Indian Institute of Technology, Kharagpur, India, December 30, 1952.

Zusammenfassung

Wenn die Aminogruppe in Zyklohexylaminen durch eine äquatoriale Bindung verknüpft ist, ist das Hauptprodukt bei der Reaktion mit salpetriger Säure das entsprechende Zyklohexanol mit einer äquatorialen Hydroxylgruppe (das heisst, die Waldensche Umkehrung findet nicht statt). Wenn die Aminogruppe durch eine polare Bindung verknüpft ist, werden beträchtliche Mengen von Zyklohexenen, begleitet von einer Mischung beider epimeren Formen des entsprechenden Zyklohexanols, gebildet (das heisst, die Waldensche Umkehrung findet statt).

[1] A. K. BOSE, Exper. *8*, 458 (1952).

The Enzymatic Hydrolysis of Glutathione by *Vibrio Cholerae*

The pathway of metabolism of glutathione, which is normally considered to be comparatively stable towards enzymes because of the γ-linkage in it, is fairly well known in animals. Thus GRASSMANN, DYCKERHOFF, and EIBELER[1] showed that oxidized glutathione was hydrolyzed into glycine and di-glutamyl cystine by pancreas. Later SCHROEDER, MUNRO, and WEIL[2] and SCHROEDER and WOODWARD[3] reported the complete breakdown of reduced glutathione into its constituent amino acids by the enzymes present in the rat kidney. The work of DYER and DU VIGNEAUD[4] on the replacement of cystine

[1] W. GRASSMANN, H. DYCKERHOFF, and H. EIBELER, Z. physiol. Chem. *189*, 112 (1930).
[2] E. F. SCHROEDER, M. P. MUNRO, and L. WEIL, J. Biol. Chem. *110*, 181 (1935).
[3] E. F. SCHROEDER and G. E. WOODWARD, J. Biol. Chem. *120*, 209 (1937).
[4] H. M. DYER and V. DU VIGNEAUD, J. Biol. Chem. *115*, 543 (1936).

by glutathione in rats held on cystine deficient diet further indicated the hydrolysis of glutathione to yield cystine or cysteine during metabolism. Besides hydrolysis, transpeptidase reactions involving glutathione and other γ-glutamyl peptides have been reported by HANES and coworkers[1] and FODER *et al.*[2].

Very little information is, however, available about the metabolism of glutathione in bacteria. So far the only role assigned to glutathione has been that of a coenzyme for glyoxylase[3]. GOULD[4] found that glutathione was essential for certain strains of gonococci after they have been cultured in the laboratory for a few weeks. In our studies on the absorption and excretion of amino acids by *Vibrio cholerae*, hydrolysis of added glutathione has been noted during growth of this organism and the observations so far made, have been reported in the present communication.

Experimental

Cholera cultures.–Ogawa 52 and Inaba 52 were used in these studies. The parent Ogawa strain was obtained from the Central Research Institute, Kasauli, and it was converted to the corresponding Inaba subtype[5].

Growth medium.–The following two media at pH 8·0 were used: (1) The glucose-salt medium reported earlier[6]. (2) The peptone medium consisting of 0·5% oxoid peptone and 0·5% sodium chloride.

15 ml of the medium were measured out in 50 ml. ERLENMEYER flasks and sterilized at 15 lbs. for 15 min. 18 h growth of *Vibrio cholerae* on papain-meat-agar slants was harvested and washed twice with saline (0·85%) and the suspension finally adjusted to a turbidity of 30% transmission, in a Lumetron Photoelectric Colorimeter (650 mμ red filter). Flasks containing the media with and without glutathione (0·03%) were inoculated with two drops of the standard bacterial suspension, incubated at 37°C for 24 and 48 h, respectively, for the peptone and glucose-salt media, and centrifuged. The cell free supernatants were used for running two phase ascending chromatograms. Uninoculated media incubated for similar periods were taken as controls.

Chromatography.–The technique of ascending chromatography, using Whatman No. 1 filter paper (size 11 ¼″ square) was adopted throughout. The chromatograms folded in the form of cylinders were kept in Pyrex glass jars of 8″ diameter and 12″ height. The solvent was kept in a petri-dish at the bottom and the jars were covered by a ground glass plate and sealed with vaseline. All experiments were conducted at room temperature (24 to 26°C). The first solvent was prepared as follows: Phenol (B.D.H.) was distilled and saturated with a salt solution containing 6·3 g sodium citrate (ANALAR) and 3·7 g of sodium or potassium dihydrogen phosphate in 100 ml of water[7]. The second solvent was n-butanol (E. MERCK), glacial acetic acid (B.D.H.) and water in the ratio of 4:1:1. The chromatograms were irrigated for about 12 to 16 h and after drying, the colour was developed by spraying with 0·1% ninhydrin in n-butyl alcohol.

Results

Since the results obtained with both Ogawa 52 and Inaba 52 were similar, only those with the former subtype are being reported.

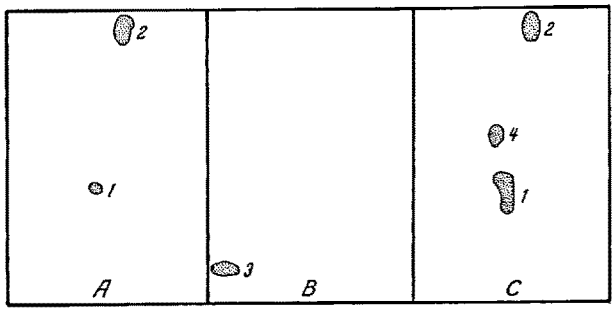

Fig. 1.—The hydrolysis of glutathione by *Vibrio cholerae* in glucose-salt medium. *1* Glutamic acid, *2* Alanine, *3* Glutathione, *4* Glycine.—*A* Supernatant after growth in glucose-salt medium. *B* Glucose-salt medium + glutathione, control. *C*. Supernatant after growth in *B*.

Hydrolysis of glutathione in the synthetic medium.-With a view to see the changes produced in the medium by the growth of *Vibrio cholerae*, control experiments were first run without the addition of glutathione. The uninoculated medium by itself, did not give any spots on the chromatograms, whereas after the addition of glutathione and incubation at 37°C, one spot (Fig. 1*B*) corresponding to it was obtained indicating that no hydrolysis had taken place. The supernatant from the growth of cholera vibrio in glutathione-free medium, gave two spots (Fig. 1*A*) which were identified to be alanine and glutamic acid. When, however, the supernatant from the growth of *Vibrio cholerae* in glutathione containing medium was chromatographed, it was found (Fig. 1*C*) that the glutathione spot had completely disappeared and the spot of glutamic acid considerably intensified. In addition, a new spot (identified as glycine) appeared on the chromatogram. In some cases glutamic acid gave a streak-like spot, almost appearing as two spots. But by running mixed chromatograms with pure acid, it was found that the two spots represented only glutamic acid. A similar chromatogram of glutamic acid has been reported recently by POLLI and BESTETTI[1].

Hydrolysis of glutathione in peptone medium.–Similar experiments when repeated in the peptone medium gave the same kinds of results (Fig. 2). A chromatogram of the uninoculated peptone medium itself, is given in Figure 2*A*. Only 8 spots appeared, out of which 3 were identified to be aspartic, glutamic and glycine. No attempt was made to identify the other amino-acids. It would be seen from Figure 2*B* that when 52 Ogawa was grown in the peptone broth, only the aspartic and glutamic acids were absorbed or metabolized from the medium, presumably as a result of deamination[2]. Addition of glutathione gave only 1 more spot in the control medium (Fig. 2*C*). This was completely hydrolyzed by *Vibrio cholerae* during growth (Fig. 2*D*). The re-appearance and intensification of the spot of glutamic acid as compared to Figures 2*B* and 2*C* respectively would indicate that the extra amount obtained from glutathione remains unmetabolised.

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[2] P. J. FODER, A. MILLER, and H. WAELSCH, Nature *170*, 841 (1952).

[3] K. LOHMANN, Biochem. Z. *254*, 332 (1932).

[4] R. G. GOULD, J. Biol. Chem. *153*, 143 (1943).

[5] D. L. SHRIVASTAVA and P. B. WHITE, Ind. J. Med. Res. *35*, 117 (1947).

[6] K. C. SAXENA, K. BHASKARAN, S. C. AGARWALA, and D. L. SHRIVASTAVA, J. Sci. Industr. Res. *12* [B], 34 (1953).

[7] Biochemical Institute-Studies IV (University of Texas Publication, Texas, U.S.A., 1951), p. 25.

[1] E. E. POLLI and A. BESTETTI, Exper. *8*, 345 (1952).

[2] A. DUDANI, S. N. IYER, C. R. KRISHNAMURTI, and D. L. SHRIVASTAVA, Current Sci. *21*, 134 (1952).

Hydrolysis of glutathione in the presence of cysteine.– Since glutathione on hydrolysis gives glutamic acid, cysteine and glycine, it was apparent from the above experiments that the cysteine liberated from the glutathione molecule was absorbed or metabolized by the

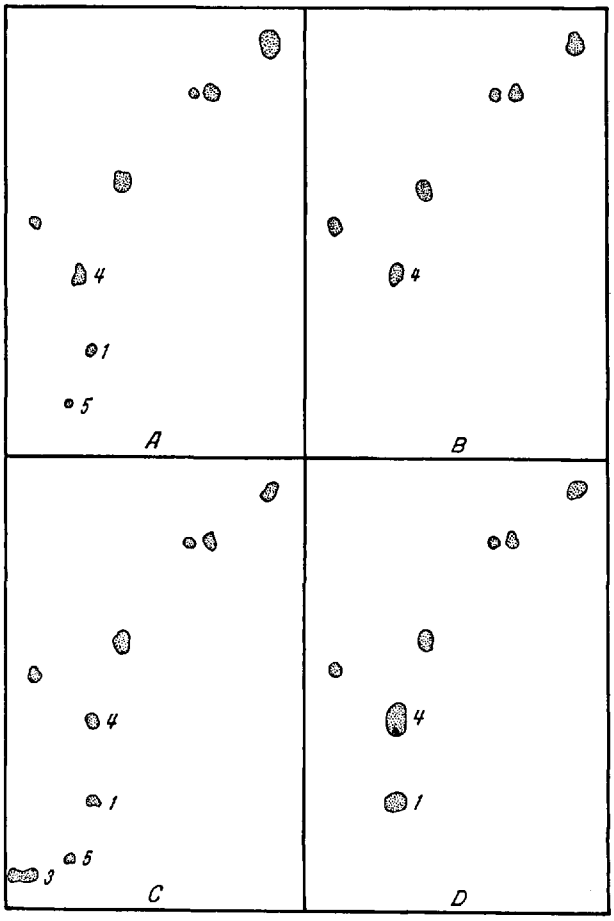

Fig. 2.—The hydrolysis of glutathione by *Vibrio cholerae* in peptone broth. *1* Glutamic acid, *3* Glutathione, *4* Glycine, *5* Aspartic acid.– *A* Peptone broth, control. *B* Supernatant after growth in *A*. *C* Peptone broth + glutathione, control. *D*. Supernatant after growth in *C*.

cells of *Vibrio cholerae*, during growth. A control experiment with the addition of cysteine in the glucose-salt medium (Fig. 3*A*) also established the fact that cysteine in the concentration of 0·03 % was completely metabolized by the growing cells of *Vibrio cholerae*, as shown by the disappearance of the cysteine spot in chromatogram 3 *B*. Even in the presence of added cysteine (Fig. 3 *C*), glutathione was hydrolyzed by the *Vibrio cholerae* cells (Fig. 3 *D*). Faint residual spot of cysteine is also seen in this chromatogram, in addition to the intense spots of glutamic acid and glycine. Alanine, of course, persists in all the chromatograms of the culture supernatants.

Discussion

Results reported above (Fig. 1*A*) in the glucose-salt medium show that growing cells of *Vibrio cholerae*, like *Bacillus anthracis*[1], *Brucella abortus*[2], and *Penicillium chrysogenum*[3] excrete alanine and glutamic acid. When glutathione, however, was added to the medium, it was hydrolyzed by the growing cells of *Vibrio cholerae* and the cysteine moiety was taken up or metabolized (Fig. 1*C*). The importance of cysteine or cystine in the growth of *Vibrio cholerae* has also been indicated by VEERARAGHAVAN[1]. It might be argued that the hydrolysis of

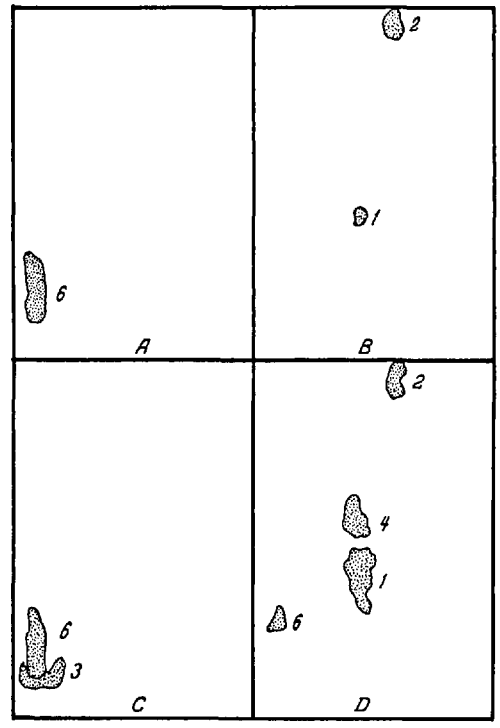

Fig. 3.—The hydrolysis of glutathione by *Vibro cholerae* in the presence of cysteine in glucose-salt medium. *1* Glutamic acid, *2* Alanine, *3* Glutathione, *4* Glycine, *6* Cysteine.—*A* Control medium + cysteine. *B* Supernatant after growth in *A*. *C* Control medium + cysteine + glutathione. *D* Supernatant after growth in *C*.

glutathione is chiefly due to the requirement of cysteine by the growing cells, but this would not appear to be true because of a similar observation (Fig. 3 *D*) in the synthetic medium irrespective of the presence of cysteine or cystine. This, along with the hydrolysis of glutathione observed in the peptone medium (Fig. 2 *D*), would point to the possible presence of a γ-peptidase in *Vibrio cholerae* cells. As far as we know, this is the first observation of the hydrolysis and metabolism of glutathione, by any pathogenic or non-pathogenic bacteria. Further work on the factors affecting the hydrolysis and its mechanism is in progress.

S. C. AGARWALA, V. K. MOHAN RAO, and D. L. SHRIVASTAVA

Central Drug Research Institute, Lucknow, India, February 1, 1953.

Zusammenfassung

1. Wachsende Zellen von Choleravibrionen geben an das Glukose und Salze enthaltende Medium Alanin und Glutaminsäure ab.

2. Dem Medium zugesetztes Glutathion wird von den Choleravibrionen hydrolysiert. Dabei treten Glykokoll und Glutaminsäure frei auf, der Zysteinrest wird im Stoffwechsel umgesetzt.

3. Es wurde gezeigt, dass die Hydrolyse des Glutathions nicht auf dem Bedürfnis der wachsenden Zellen an Zystein beruht.

4. Es wird angenommen, dass die Zellen der Choleravibrionen eine γ-Peptidase besitzen.

[1] R. D. HOUSEWRIGHT and C. B. THORNE, J. Bact. *60*, 89 (1950).
[2] R. J. GOODLOW, W. BRAUN, and L. A. MIKA, Arch. Biochem. *30*, 402 (1951).
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[1] N. VEERARAGHAVAN, Nature *163*, 138 (1949).